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(54) Title: PROCESS FOR THE PRODUCTION OF COMBINATORIAL COMPOUND LIBRARIES

(57) Abstract

The present invention relates to a process for the preparation of a plurality of different units consisting of a solid or semisolid carrier (bead), a synthetic oligomer (ligand) and an identification structure (tag) by means of which the monomers of the ligands are coded, and the use of said library for searching for novel classes of compounds and individual compounds. The invention further relates to compounds found with the novel process and the use thereof as thrombin inhibitors.

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Process for the production of combinatorial compound libraries

The present invention relates to a process for the preparation of a plurality of different units consisting of a solid or semisolid carrier (bead), a synthetic oligomer (ligand) and an identification structure (tag) encoding the monomers of the ligands, and to the use of said library for searching for novel classes of compounds and individual compounds. The invention further relates to compounds detected with the novel process and to the use thereof as thrombin inhibitors.

Recently there has been an ever-increasing demand for chemical compounds that bind selectively only to a specific receptor. These compounds can then be used, inter alia, as inhibitors, agonists, antagonists, or for marking. In this connection, the possibility has been developed of simultaneously synthesising a multiplicity (library) of different compound and assaying them for their ability to bind to a specific receptor. These libraries (combinatorial compound libraries =CCL) usually consist of natural amino acids or of nucleotides and, ever more frequently, also of modified amino acids, modified nucleotides and other chemical compounds which are used as monomer building blocks.

The individual different ligands of these libraries are normally synthesised in parallel, either as mixtures or as physically separated individuals. These parallel syntheses make it possible to produce libraries containing a very large number of different ligands over a shorter period of time.

The literature describes two intrinsically different main strategies for the chemical synthesis of libraries consisting of a plurality of test candidates.

1. The ligands are obtained after their synthesis together in solution (Houghten *et al.* Nature (1991) **354**, 84-86).
2. The ligands are synthesised on a solid carrier, the synthesis being controlled such that only one species of ligand is bound to the carrier (one bead one sequence; Lam *et al.* Nature (1991), **354**, 82-84; Furka *et al.*, 14th Intern. Congr. Biochem. FR013, 1988).

In the first strategy the problem consists in clearly identifying the desired ligand, as all ligands are present simultaneously in the solution. To identify ligands with a number of variable positions, it is necessary to synthesise a pool for each possible monomer per variable position, so that for a ligand having 4 variable positions, each of which may be

occupied by one of 20 amino acids, 4×20 = 80 pools have to be synthesised and assayed.

The second strategy produces all desired possibilities in a single pool and permits simple separation of the solid carriers containing the desired ligands and hence the identification of the ligands. The limitation here, however, resides in the type of ligands used, as their composition must subsequently be identified. Accordingly, only ligands are suitable that can be clearly identified by sequencing (peptides from naturally occurring amino acids, DNA and RNA). A further drawback is that the attachment of the ligands to solid carriers can lead to false-positive results, i.e. many assays cannot be carried out with ligands that are attached to solid carriers.

In contrast to these known possibilities, the present invention provides a library in which to each solid carrier only one type of ligand (one bead, one sequence) and one identification structure coding for this ligand (tag) is bound. The clear assignment of one ligand to one tag makes it possible to identify in a binding assay also those ligands which, in the amounts in which they are present on a carrier, cannot be unequivocally identified by known methods and/or by automated procedures. A further surprising advantage is that the ligand can be separated from the carrier for a further assay without loss of identifiability in its entirety or in portions, so that the results of the assay are in no way influenced by the solid carrier. With the identified ligands that are present in homogeneous solution it is thus possible to carry out assays which are not possible with the ligands bound to the carrier, for example further enzymatic assays or purity assays by IR, NMR or MS.

Accordingly, the invention relates to a library comprising a plurality of different units, each consisting of a solid or semisolid carrier (beads), a synthetic oligomer (ligand) and an identification structure (tag) by means of which the monomers of the ligand can be indentified, wherein:

a) each carrier unit carries only one type of ligand,
b) ligand and tag are attached to the same carrier at different positions,
c) the ligand, irrespective of the tag and without altering its information content, can be separated from the carrier,
d) the tag is a sequenceable polypeptide, and
e) to synthesise the tag or ligand a protective group is used that can be removed under mild acid conditions.

It is thus possible by means of the inventive process to carry out two different assays:

a) a first assay to preselect the ligand bound to the acceptor, said ligand being bound to the solid or semisolid carrier,

b) a second assay, after separation of the ligand from the carrier and the information structure, for the further characterisation of the ligand. The second assay can likewise investigate the attachment of the ligand to the acceptor or can be a completely different assay which cannot be carried out with the attached ligands.

Protective groups which can be removed under mild acidic conditions can be, for example, completely removed at concentrations of up to 10 %, preferably of up to 5 %, of acetic acid. These preferred protective groups will be at least 10x more labile than the dimethyl-dimethoxybenzyloxycarbonyl protective group (Ddz). Particularly preferred groups are those of the trityl type, such as unsubstituted or alkoxy-substituted trityl.

The term ligand embraces all compounds specifically comprising a plurality of monomers. Typically the monomers contain at least two reactive groups. Illustrative examples of such reactive groups are amino, azido, isocyanido, isocyanato, hydrazino, carbonyl, carboxyl, acylhalogeno, hydroxyl, sulfhydryl, sulfonyl chloride, phosphate groups, and halogeno groups. To facilitate the synthesis, the reactivity of the groups can be modified by protective or activating groups. Typical examples of monomers containing two or more reactive groups are natural and non-natural amino acids, ω-aminocarboxylic acids, saccharides, nucleotides and nucleotide analogs. When using monomers containing more than two reactive groups it is also possible to insert optional branches and cyclisations of the ligand. Monomers containing only one reactive group can be used as end groups.

The inventive process characterised in detail hereinbelow also makes it possible to produce libraries of ligands in which the individual building blocks are linked not only in succession, but also side by side, in a specific combination to a basic compound. This can be done by attaching a basic building block that contains a number of reactive groups direct or through a linker to the carrier and then attaching to this basic building block individual monomers that in turn contain one or more than one reactive group. Basic building blocks in the context of this invention are typically steroid nuclei, the penicillin or penem nucleus, soraphens, benzodiazipines, saccharides or desferoxiamine. Different reactive groups can then be fixed to these nuclei by standard chemical methods.

Preferred ligands contain monomers that cannot be identified clearly by sequencing

without further experimental effort, e.g. by known automated procedures.

Monomers that are not clearly identifiable by sequencing are all chemical compounds except the naturally occurring 20 amino acids and the nucleotides naturally occurring in DNA and RNA. Typical examples are modified amino acids or nucleotides, ω-aminocarboxylic acids, D-amino acids, saccharides, amino acids having saccharide side-chains, and end groups carrying only one reactive group such as acetyl or benzyl.

The ligand is usually attached to the solid or semisolid carrier through a linker. Linkers for the ligand are typically chemical compounds containing at least two reactive groups. Preferred linkers are those that tolerate mild basic as well as mild acidic conditions, so that ligands and tags can not only be synthesised but also assayed on the carrier. The preferred linkers are therefore cleavable only by means of a specific reaction, for example methionine, which is cleavable with cyanogen bromide, or linkers which are cleavable only under strongly basic or strongly acidic conditions, photolytically, or under reductive or oxidative conditions. Particularly preferred linkers are those that form cleavable bonds under basic, but stable bonds under acidic, conditions, so that the ligand can be removed selectively from the carrier. Illustrative examples of suitable linkers are p-hydroxymethyl-benzoic acid, 4-hydroxymethylphenylacetic acid, benzhydrylamino, allyl, hydroxycroto-nylaminomethyl, 3-nitro-4-hydroxymethylbenzoic acid, p-nitrobenzhydrylamine, 4-[4,4'-bis(methylsulfinyl)-2-oxybenzhydrylamino]butyric acid and disulfide linkers that in turn can be linked through a group called in solid-phase synthesis a "handle". These linkers suitable for use in the inventive process may contain, in addition to the described cleavable building blocks, further building blocks that influence or do not influence the cleavage reaction, which are suitable for separating the variable part of the ligand spatially from the cleavable group.

Short peptides which have a specific site for a protease such as trypsin, yscα, yscF or the V8-protease may also be used as linkers.

Particularly preferred linkers are also those whose cleavage can be controlled, so that under suitable conditions only a specific part of the linker is cleaved, thereby affording the possibility of separating the ligands from the solid carrier in several portions, e.g. for further assays. Those linkers are very particularly preferred that can be cleaved by a volatile or gaseous agent such as ammonia, as these linkers - before the assay of the ligands - can be removed completely or almost free of residue.

To be able to cleave the different ligands in exactly defined and - despite the heterogeneity of the ligands - equal portions, it is possible for the linker between the carrier and the ligand to consist of a cleavable part, as indicated above, as well as a part which is the same for all ligands. Suitable building blocks of the constant part may be in general chemical compounds which permit attachment not only to the cleavable part of the linker, but also to another chemical building block or to the ligand. Illustrative examples are bifunctional chemical compounds such as amino acids. nucleic acids or invariable parts of the ligand.

The information structure (tag) is a sequenceable polypeptide whose coding units conveniently consist of the naturally occurring amino acids (Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val). It is preferred to use amino acids which carry no reactive side-groups and/or are readily identifiable during sequencing, typically Ala, Asn, Asp, Gly, Ile, Leu, Phe, Pro, Trp and Val. Particularly preferred amino acids are Ala, Asn, Asp, Gly, Leu, Phe and Pro. The tag can in turn be bound to the carrier through one of the above described linkers which is cleavable under specific conditions. It is important in this connection that the separation of the linker and the tag is possible independently of the other and that the separation of the ligand from the carrier does not modify the information of the tag.

If all 20 naturally amino acids are used, then it is possible to code 20 different monomers per variable site. If it is desired to encode more than 20 different monomers per variable site, then more than one amino acid is used for each coding unit. By using dipeptides it is thus possible to code up to 20^2 different monomers with 20 different amino acids.

To avoid misinterpretations when sequencing the tag, it is possible to use only the preferred amino acids listed above. These amino acids can then be used as di- or tripeptides to increase the number of codable monomers, i.e. each monomer of the ligand is coded by more than one amino acid in the tag.

In order to ensure that the tag influences the test result as little as possible and that the amount of ligand is as high as possible, the ratio of ligand to tag on a solid or semisolid carrier is desirably greater than 1, preferably from 2 to 100 and, most preferably, from 4 to 10.

For practical considerations, the tag can be provided with a starting and/or end sequence

that does not code for a monomer of the ligand, so that, when sequencing the tag, it is possible to identify exactly the beginning and end of the part coding for the ligand. Illustrative examples of such starting and end sequences are amino acids that do not code for a monomer of the ligand as well as di- or tripeptides of identical amino acids such as Val-Val or Leu-Leu-Leu.

The term "solid or semisolid carrier" will be understood as meaning macroscopic particles that are insoluble in the reaction media and to which both the ligand and the tag can be bound in sufficient amount. The amount of tag that can be bound to the carrier must be sufficiently large to produce a clearly identifiable signal (e.g. > 1 pmol) when sequencing the tag. If the tag contains radioactively marked monomers, then also less than 1 pmol of tag per carrier can be identified on account of the lower detection limit for radioactive compounds.

The binding of ligand and tag is effected by means of reactive groups at the surface of the carrier, e.g. amino, carboxyl, hydroxyl or halogen groups. These reactive groups are usually already constituents of the carrier, but they can also be applied or modified subsequently. The resins customarily employed in solid-phase synthesis can be used, for example those used in Merrifield peptide synthesis. They consist largely of a polystyrene molecule that is crosslinked by copoylmerisation with divinyl benzene. The molecules are additionally derivatised to attach the reactants in the solid-phase synthesis.

The invention also relates to a process for the preparation of the above described library, which comprises synthesising ligand and tag by an orthogonal and alternating synthesis.

The synthesis of the libraries of this invention comprises the steps

a) attaching the first unit of the tag (preferably in less than equivalent amount, i.e. less than 50 mol % based on the reactive groups on the carrier) and the first monomer of the ligand or the building block thereof or a linker for each to the solid or semisolid carrier;
b) optionally attaching further non-variable monomers to the ligand or further none-variable coding units to the tag;
c) dividing the solid or semisolid carrier into portions for the variable monomers of the ligand;
d) carrying out in each portion separately, in codable sequence, further modifications at the ligand or attaching another of the variable monomers possible at this site of the ligand as well as the unit of the tag coding for this step;

ligand as well as the unit of the tag coding for this step;

e) mixing the portions;

f) repeating steps b) to e) until the variable part of the ligand is completely synthesised; and

g) optionally attaching one or more than one further invariable monomer to the ligand or further non-coding units to the tag (q.v. reaction schemes 1 and 2);

using for the synthesis of tag or ligand a protective group which can be removed under mild acidic conditions.

The type of the bond of the first unit of the tag and of the first monomer of the ligand and the linker for the first monomer depends on the type of chosen reactive group and is effected by the standard methods for such groups.

The further synthesis is carried out by standard known methods of solid-phase synthesis (Fields *et al.*, Intern. J. Pept. Prot. Res. (1990), **35**, 161-214). The transient protective groups (protective end groups which can be eliminated before each synthesis step) used for the synthesis of ligand and tag are orthogonal protective groups, i.e. the syntheses of ligand and tag can be carried out independently of each other. For example, two groups of protective groups can be used, the first group being removable under mild acidic and the other group under basic conditions, by the action of light, under oxidative or reductive conditions. It is particularly preferred to use a combination of protective groups that are removable under mild acidic conditions and protective groups that are removable under basic conditions.

To ensure compatibility with a large number of protective groups which shall remain intact during the ligand synthesis, the acid-removable transient protective groups are those having especially pronounced acid lability. Suitable acid-labile protective groups are at least 10x more labile than the dimethyldimethoxybenzyloxycarbonyl protective group (Ddz).

To achieve full orthogonality, base-removable fluorenylmethoxycarbonyl (Fmoc) is preferably used for the synthesis of the one structure, and acid-removable trityl (Trt) or substituted trityl protective groups such as alkoxy-substituted trityl for the synthesis of the other structure.

Illustrative examples of the combination of protective group types are:

Ligand	Tag	Side-chains of the monomers
Fmoc type	Trt type	acid-labile or allyl type
Trt type	Fmoc type	acid-labile or allyl type
allyloxycarbonyl	Trt type	acid-labile
Trt type	allyloxycarbonyl	acid-labile
Trt type	Boc or NPS type	allyl type
Boc or NPS type	Trt type	allyl type
allyloxycarbonyl	Trt type	β-elimination type
Trt type	allyloxycarbonyl	β-elimination type

Groups of the β-elimination type are typically protective groups of the fluorenylmethyl type; NPS denotes groups of the nitrophenylsulfenyl type. A general description of the eligible groups will be found in Fields *et al.* (Intern. J. Pept. Prot. Res. (1990), **35**, 161-214).

Also suitable are combinations of one of the above groups of protective groups for ligands or tags with protective groups which can be removed by photolysis, e.g. nitrobenzyl or nitroveratryloxycarbonyl groups.

The reaction steps required e.g. for the synthesis of amides are widely known in the art and usually depend on the type of activation of the carboxylic acid group participating in the reaction. The reactions normally run in the presence of a condensing agent or, when activating the carboxylic acids in the form of anhydrides, of an agent that binds the carboxylic acid formed. In some cases it is also possible to add chaotropic reagents such as LiF in NB-methylpyrrolidone. The reactions are carried out in the temperature range from -30°C to +150°C, preferably from +10°C to +70°C and, most preferably, from +20°C to +50°C, and, if appropriate, also in an inert gas atmosphere.

Illustrative examples of useful condensing agents are carbodiimides such as N,N'-diethyl-, N,N'-diisopropyl-, N,N'-dicyclohexyl- or N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide; carbonyl compounds such as carbonyl diimidazole; 1,2-oxazolium compounds such as 2-ethyl-5-phenyl-1,2-oxazolium-3'-sulfonate and 2-tert-butyl-5-methylisoxazolium perchlorate; acylamino compounds such as 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline; and uronium compounds such as 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetra-

methyluronium tetrafluoroborate (TBTU); or phosphonium compounds such as benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) or benzotriazol-1-yl-oxy-pyrrolidinophosphonium hexafluorophosphate (PyBOP).

Useful acid acceptors are typically alkali metals, carbonates or bicarbonates, such as sodium or potassium carbonate or bicarbonate (usually together with a sulfate), or organic bases such as sterically hindered tri-lower alkylamines such as N,-diisopropyl-N-ethylamine.

Reactive side-chains of the monomers of the ligand and tag which shall not participate in the reactions may be protected by a third group of protective groups. Useful protective groups and processes for their introduction and removal are described, inter alia, in "Protective Groups in Organic Chemistry", Plenum Press, London, New York 1973; "Methoden der organischen Chemie", Houben-Weyl, 4th edition, Vol. 15/1, Georg-Thieme-Verlag, Stuttgart 1974; Th. W. Greene, "Protective Groups in Organic Synthesis", John Wiley & Sons, New York 1981; Atherton *et al.*, "Solid phase peptide synthesis-A practical approach" IRL Press Oxford University, 1984; Jones, "The chemical synthesis of peptides", Oxford Science Publications, Clavendon Press Oxford, 1991; und Bodanszky, "Peptide Chemistry", Springer Verlag Berlin, 1988.

Typical examples of hydroxy protective groups are acyl radicals such as the tert-butoxycarbonyl radical, etherifying groups such as the tert-butyl group, and silyl or tin radicals such as the tri-n-butyltin radical or tert-butyldimethylsilyl.

Carboxyl groups may be protected by ester formation with groups of the tert-butyl type, benzyl, trimethylsilylethyl or 2-triphenylsilyl groups.

Amino groups may conveniently be protected by removable acylamino, arylmethylamino, esterified mercaptoamino, 2-acylalk-1-enylamino, silylamino, tin amino or azido groups, including tert-butoxycarbonyl, allyloxycarbonyl, benzyloxycarbonyl, 4-nitrobenzyloxycarbonyl, diphenylmethoxycarbonyl, nitrophenylsulfenyl, 2,2,2-trichloroethoxycarbonyl, pentamethylchromanosulfonyl (PMC) or methoxytrimethylbenzylsulfonyl (Mtr) protective groups.

Thiols may be protected by acetamidomethyl groups.

These protective groups are usually removed after the complete synthesis of ligand and tag jointly by conventional methods of peptide chemistry, conveniently by treatment with 95 % trifluoroacetic acid. In some cases strong nucleophiles, such as 1,2-ethanedithiol, may additionally be added to capture the generated protective groups.

Further methods of removing these protective groups are known and comprise, inter alia, β-elimination, solvolysis, hydrolysis, alcoholysis, acidolysis, treatment with a base or reduction.

The number of portions for the reactions described in d) preferably corresponds in each case to the number of possibilities of the next monomer of the ligand to be attached. As stated above, one unit of the tag can consist of one or more than one amino acid. If a unit consists of several amino acids, each of these can be attached in succession to the growing tag or synthesised separately as unit and then attached in a reaction to the tag. Sequencing of the tag can be made easier by additionally attaching to the beginning and end thereof one or more than amino acid which indicates the start or end of the part carrying the information (start sequence/end sequence).

To identify the ligand which binds to the desired acceptor, the library prepared above is treated with the acceptor to be investigated, and those carriers to which the acceptor remains attached are washed and isolated.

Possible acceptors within the scope of this invention are macromolecular units which have affinity for binding to one or more than one ligand. Illustrative examples are receptors including serotonin receptor, GABA receptor and benzodiazepine receptor; transport proteins such as Na and K channels, antibodies and enzymes such as proteases, thrombin, renin, ACE, aromatase and reverse transcriptase; or fragments thereof which have the same binding properties for the ligands. The ligands binding to these acceptors act as antagonists, inhibitors, agonists, or as markers for the acceptors.

Unless not already naturally present, the acceptors are provided with an identifiable group such as a fluorescing, chemoluminescing or radioactive group, avidine, biotine, reporter enzymes, immunologically detectable groups (ELISA) or the like. Methods of marking with the markers referred to above and of affinity chromatography are generally known in the art.

The isolation of the carriers to which an acceptor is bound is effected manually by sorting the carriers, e.g. under UV or blue light in the case of fluorescing acceptors, or with the aid of automated apparatus typically used for sorting cells.

The tag of each carrier isolated in this manner can be sequenced with the conventional methods of peptide analysis, e.g. Edman degradation, and the ligand attached to said carrier clearly identified. Sequencing can be carried out before or after separation of the ligand, e.g. by an automated sequencing apparatus, at the tag attached to the carrier. Sequencing after separation of the ligand is preferred, as in this case liberated monomers of the ligand do not interfere with the sequencing. Another possibility consists in derivatising the ligands terminally in suitable manner (e.g. by acetylation), so that they are not degraded by the analysis of the tag.

A further possibility consists in separating the tag from the carrier and then sequencing the tag.

To rule out false-positive results of the binding test, the ligands can be separated wholly or partially from the individual carriers and, in homogeneous solution, subjected to a further assay, e.g. a second round of assays for their attachment to the acceptor. The separation of the ligand is effected e.g. by cleavage at the linker between the carrier and the ligand by a reaction specific to said linker. If the linker is p-hydroxybenzoic acid, this cleavage can be effected by treatment of the individual carriers with gaseous ammonia, a saturated solution of ammonia/THF or liquid ammonia (e.g. in a microtitration plate). The separated ligand can then be rinsed from the carrier and once more tested for its bonding to the acceptor, or a further assay is carried out which cannot be performed with the ligands bound to the carrier, typically binding studies, tests in which light absorption is measured, or MS, IR or NMR investigations.

A suitable choice of linker also makes it possible, for a first assay, to separate a first portion of the ligands from the isolated carriers by treatment with gasesous ammonia, and a second portion of the ligands for a further assay by treatment with a solution of ammonia/THF or liquid ammonia. A suitable linker in this connection is typically 4-hydroxymethylbenzoic acid.

A typical example is the synthesis of a library of potential thrombin inhibitors which contain non-natural amino acids and other building blocks and are thus more

protease-resistant.

The library consists e.g. of pentamers having 2 constants and 3 variable positions and having the composition:

X - Y - Z - Pro - GABA

X= 2-cyanobenzenesulfonyl, D-Phe, N-benzylglycine, β-Ala or acetyl
Y= L-Pro, D-Pro, β-Ala or L-Asp
Z= L-Arg, D-Arg, β-Ala, L-Asp or sarcosyl

This composition affords 5×4×5 = 100 possibilities for the composition of the ligands.

The individual monomers are coded by dipeptides in the identification structure.

After screening this library, it is found that the pentamers D-Phe-D-Pro-Arg-Pro-GABA and D-Phe-Pro-D-Arg-Pro-GABA are all effective thrombin inhibitors.

Accordingly, a further object of the invention is a compound of formula D-Phe-D-Pro-Arg-Pro-GABA, D-Phe-Pro-D-Arg-Pro-GABA, or pharmaceutically acceptable salts thereof, for inhibiting thrombin; and pharmaceutical compositions that contain said thrombin inhibitors, singly or in combination with further optional excipients.

The invention further relates to each compound detected by the novel process for use in a method of therapeutic and prophylactic treatment of the human or animal body. The preferred field of use is that of therapeutic and prophylactic treatment in connection with the thrombin function such as embolisms and thromboses.

The pharmaceutical compositions of this invention are those for enteral, e.g. oral, and also rectal and parenteral administration, for example subcutaneous, intravenous or intraperitoneal administration, to warm-blooded animals, and they contain the pharmacologically active compound alone or together with a pharmaceutically acceptable carrier. The daily dose will depend on the age and individual condition of the patient as well as on the mode of administration.

The novel pharmaceutical compositions contain from about 10 to 80 %, preferably from about 20 to 60 %, of the active compound. Pharmaceutical compositions for enteral or parenteral administration are typically those in dosage unit forms such as dragées, tablets, capsules or suppositories, and also ampoules. These dosage forms are prepared in a manner known per se, typically by conventional mixing, granulating, confectioning, dissolving or lyophilising methods.

Pharmaceutical compositions for oral administration are preferred. Suitable carriers are especially fillers such as sugars, conveniently lactose, saccharose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, typically tricalcium phosphate or calcium hydrogen phosphate, and also binders such as starch pastes, conveniently using maize, corn, rice or potato starch, gelatin, tragacanth, methyl cellulose and/or polyvinyl pyrrolidone, and/or, if desired, disintegrators such as the above-mentioned starches, also carboxymethyl starch, crosslinked polyvinylpyrrolidone, agar, alginic acid or a salt thereof such as sodium alginate. Excipients are in particular glidants, flow control agents and lubricants, conveniently silica, talcum, stearic acid or salts thereof, typically magnesium stearate or calcium stearate, and/or polyethylene glycol. Dragée cores can be provided with suitable non-enteric or enteric coatings, typically using concentrated sugar solutions which may contain gum arabic, talcum, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, shellac solutions in suitable organic solvents or mixtures of solvents or, for the preparation of enteric coatings, solutions of suitable cellulose preparations such as acetyl cellulose phthalate or hydroxypropylmethyl cellulose phthalate. Dyes or pigments can be added to the tablets or dragée coatings, conveniently to identify or indicate different doses of active compound.

Further pharmaceutical compositions for oral administration are dry-filled capsules made of gelatin and also soft-sealed capsules consisting of gelatin and a plasticiser such as glycerol or sorbitol. The dry-filled capsules can contain the active ingredient in the form of granules, conveniently in admixture with fillers such as lactose, binders such as starches, and/or glidants such as talcum or magnesium stearate, and with or without stabilisers. In soft capsules, the active ingredient is preferably dissolved or suspended in a suitable liquid, typically a fatty oil, paraffin oil or a liquid polyethylene glycol, to which a stabiliser can also be added.

Suitable pharmaceutical compositions for rectal administration are typically suppositories, which consist of a combination of the active compound with a suppository base. Examples

of suitable suppository bases are natural or synthetic triglycerides, paraffin hydrocarbons, polyethylene glycols and higher alkanols. It is also possible to use gelatin capsules for rectal administration that contain a combination of the active compound with a base substance. Suitable base substances are typically liquid triglycerides, polyethylene glycol or paraffin hydrocarbons.

Pharmaceutical compositions for parenteral administration contain aqueous solutions or suspensions of the active compound, conveniently oily injection suspensions using suitable lipophilic solvents or vehicles such as fatty oils, typically sesame oil, or synthetic fatty acid esters such as ethyl oleate or triglycerides, or aqueous injection suspensions which may contain viscosity increasing substances, conveniently sodium carboxymethyl cellulose, sorbitol and/or dextran, and also with or without stabilisers.

The invention also relates to the use of the above mentioned thrombin inhibitors, preferably in the form of pharmaceutical compositions. The dosage of the active compound will depend on the species of the warm-blooded animal, on the age and individual condition of the patient, and also on the mode of administration. The contemplated daily dosage for parenteral administration to a patient of approximately 75 kg body weight will be from about 0.1 mg to 500 mg, preferably from about 1 mg to 50 mg.

The invention is illustrated in more detail by the following Examples.

The following abbreviations are used:

DCCI = dicyclohexylcarbodiimide
DCE = dichlorethane
DICD = diisopropylcarbodiimide
DIPEA = diisopropylethylamine
DMA = dimethylacetamide
DMF = dimethylformamide
DMAP = dimethylaminopyridine
DMSO = dimethylsulfoxide
Fmoc = fluorenylmethyloxycarbonyl
GABA = γ-aminobutyric acid
HOBT = hydroxybenzotriazole

NEt_3 = triethylamine
OSu = O-succinimide
Pip = piperidine
PMC = pentamethylchromanesulfonyl
Trt = trityl

Repetitive treatments are carried out in accordance by the batch process with the procedures of the solid-phase technique commonly used in peptide chemistry (Fields *et al.*, Intern. J. Pept. Prot. Res. (1990), **35**, 161-214), e.g. by addition of reagent solutions to the resin and subsequent filtration (isolation of the filtrate under a weak vacuum).

The preparation of a library (q.v. reaction scheme 1), consisting of pentamers having 2 constant and 3 variable positions and having the composition

X - Y - Z - Pro - GABA

X = 2-cyanobenzenesulfonyl, D-Phe, N-benzylglycine, β-Ala, acetyl
Y = L-Pro, D-Pro, β-Ala, L-Asp
Z = L-Arg, D-Arg, β-Ala, L-Asp, sarcosyl

is described

The individual monomers described above are coded by the following dipeptides in the identification structure:

Building blocks	Dipeptide
2-cyanobenzenesulfonyl chloride	DG
D-Phe	GD
N-benzylglycine	GF
β-Ala	AA
Acetyl	AG
L-Pro	DA
D-Pro	GA
L-Arg	GG

D-Arg	NG
L-Asp	AN
sarcosyl	NA

Example 1: Preparation of the trityl-protected amino acids and dipeptides

10 mmol of each dipeptide or amino acid are suspended in 36 ml of chloroform/acetonitrile (4:1). Then 10 mmol of trimethylsilyl chloride are slowly added dropwise, and the reaction mixture is refluxed for 2 hours at c. 65°C. Then 20 mmol of triethylamine are added dropwise, and finally a solution of 10 mmol of trityl chloride in 10 ml of chloroform is added. After 60 minutes methanol is added in excess and, after a further 5 minutes, the reaction mixture is concentrated by evaporation and the residue is dried. The crude material is taken up in an ice-cooled 2 M solution of potassium hydrogensulfate (or citric acid) and the solution is extracted with ethyl acetate. After back-extraction with aqueous alkali (at c. 5°C), the aqueous phase is adjusted with potassium hydrogensulfate solution to pH 6-7. The Trt-dipeptide or the Trt-amino acid precipitates and is extracted with ethyl acetate. 10 mmol of triethylamine are added to the organic phase and the batch is concentrated by evaporation (triethylammonium salt of Trt-dipeptide or Trt-amino acid),

The triethylammonium salts of the Trt-dipeptides DG, GD, GF, AA, AG DA, GA, GG, NG, AN and NA as well as the Trt-amino acids of A, D, F, G and N are prepared in this manner.

Example 2: Partial derivatisation of the carrier resin with a less than equivalent amount of a constant unit of the tag

181 µmol of reactive alkylamino groups of a Polyhipe SU 500® resin (Novabiochem), derivatised with ethylenediamine, are each washed 5x for 40 seconds with 20 % Pip/DMA and afterwards 10x for 60 seconds with DMA at room temperature.

Loading: c. 430 µmol/g are equivalent to c. 2000 pmol/resin particles (bead)

For preactivation, 60 µmol of Trt-Gly-OH·NEt_3 of Example 1 are dissolved in 66 µmol (based on HOBT) of a 0.5 M solution of HOBT/DMA and to this solution are added 66 µmol (based on DICD) of a 2 M solution of DICD/DMA. After 40 minutes at room temperature, the mixture is diluted with 2 ml of DMA and the solution is pipetted onto the resin. Immediately afterwards, 66 µmol of DIPEA are added. The coupling reaction is continued for 60 minutes. The resin is rinsed 10x 45 seconds with DMA.

Example 2.1: Determination of the tag loading by measuring the free amino groups
The reporter group Fmoc is coupled to the resin and, after washing off excess amounts, split off again and measured.

Procedure:
275 µmol of Fmoc-OSu are dissolved in 2.1 ml of DMA and added to the resin. Immediately afterwards, 413 µmol (based on DIPEA) of a 1.5 M solution of DIPEA/DMA are pipetted onto the resin. After 80 minutes at room temperature, the resin is washed 10x 18 seconds with DMA. To remove the Fmoc group, the resin is treated 15x 40 seconds at room temperature with 20 % of Pip/DMA. The collected filtrates are diluted in defined manner and their extinction is measured at 299.8 nm. The total number of free amino groups is c. 154 µmol, i.e. 85 % of the total loading of the resin (percentage of ligand). The tag loading of the resin is thus c. 15%.

Example 3: Coupling the linker for the ligand to the resin
For preactivation, 769 µmol of 4-hydroxymethylbenzoic acid are dissolved in 845 µmol (based on HOBT) of a 0.5 M solution of HOBT/DMA and to the solution are added 845 µmol (based on DICD) of a 2 M solution of DICD/DMA. After 40 minutes at room temperature, the reaction mixture is diluted with 0.3 ml of DMA and pipetted onto the resin. Immediately afterwards, 845 µmol of DIPEA are added and the coupling reaction is continued for 60 minutes at room temperature. Afterwards rinsing is effected is follows:

10x 45 seconds with DMA
5x 60 seconds with isopropanol
6x 45 seconds with DMA

Example 4: Linking the first constant C-terminal component of the ligand with the linker

Batch: 154 µmol
The modified resin of Example 3 is pipetted to a solution of:

615 µmol	of Fmoc-GABA-OH
600 µl	of DMA and
3 ml	of dichloroethane

To this suspension is added a solution of:

646 µmol	of DCCI and
307 µl	of dichloroethane

in 2 portions over 5 minutes at room temperature and, finally, a solution of:

30.7 µmol of DMAP and

65 µl of dichloroethane

is added. After a total time of 20 minutes, 154 µmol of N-methylmorpholine are added. The reaction is continued for 4 hours and afterwards rinsing is effected as follows:

4x 45 seconds with DMA

3x 45 seconds with dichloroethane

4x 45 seconds with DMA.

Example 5: Chain lengthening of the constant part of the ligand

The Fmoc group is removed, as described in Example 2. Measurement of the extinction gives an amount of ligand of 122 µmol.

For preactivation, 367 µmol of Fmoc-Pro-OH are dissolved in 404 µmol (based on HOBT) of a 0.5 M solution of HOBT/DMA and to this solution are added 404 µmol (based on DICD) of a 2 M solution of DICD/DMA. After 40 minutes at room temperature, the reaction mixture is diluted with 1.3 ml of DMA and added to 122 µmol of resin of Example 4. Immediately afterwards, 404 µmol of DIPEA are added, and the coupling reaction is continued for 45 minutes at room temperature. A post-coupling with the same amount of Fmoc-Pro (as above) is carried out to increase the yield (another 35 minutes). The resin is rinsed and treated 1x 4 minutes with acetic anhydride/pyridine/DMA 1:1:8 (v:v:v) to block unreacted amino groups and subsequently rinsed as follows:

5x 45 seconds with DMA

5x 45 seconds with isopropanol.

The resin is dried and portioned into suitable pools for introduction of the variable positions. The introduction of the variable Z in this Example requires 5 portions for the separate reactions with 5 different building blocks ("split synthesis").

Example 6: Synthesis of the variable parts of the ligands and of the corresponding tag

Portioning into 5 pools each of 19 µmol is carried out (one pool for each variable possible at this position).

Example 6.1: Synthesis of the 1st variable position of the ligand

First the Fmoc group is removed at the ligand as described in Example 2. Then follow the separate coupling reactions for the 1st variable position (Z position):

Pool	Monomer	Amount	Coding
1	Fmoc-L-Arg(PMC)-OH	76 µmol	GG
2	Fmoc-D-Arg(PMC)-OH	76 µmol	NG
3	Fmoc-β-Ala-OH	76 µmol	AA
4	Fmoc-L-Asp(OtBu)-OH	76 µmol	AN
5	Fmoc-sarcosyl-OH	76 µmol	NA

Each of the above listed Fmoc building blocks is preactivated with 84 µmol of HOBT solution (0.5 M in DMA) and 84 µmol of DICD solution (2 M in DMA) for 40 minutes at room temperature and, after dilution with 230 µl of DMA, added to one resin portion. Immediately afterwards, 84 µmol of DIPEA are added. The coupling is carried out for 45 minutes at room temperature. A post-coupling is carried out for an additional 35 minutes with an identical mixture. Acetylation and rinsing are carried out as described in Example 5.

Example 6.2: Chain lengthening of the tag via dipeptides

In accordance with the code table, in each pool of Example 6.1, in accordance with the coupled monomer, the tag portion with the Trt peptide of Example 1 coding for the monomer is individually further synthesised (q.v. reaction scheme 2).

First the trityl groups of the tag irreversibly linked to the resin are removed by cleavage with 5 % formic acid (2x for 1 minute) in dichloroethane and rinsed:

5x 45 seconds with DMA
3x 45 seconds with 3 % triethylamine in DMA
3x 45 seconds with DMA.

Afterwards the dipeptides are linked to the respective tags in separate reactions.

1	Trt-GG	129 µmol
2	Trt-NG	129 µmol
3	Trt-AA	129 µmol
4	Trt-AN	129 µmol
5	Trt-NA	129 µmol

The above listed Trt-dipeptides are preactivated for c. 40 minutes in 142 µmol of HOBT and 142 µmol of DICD solution in DMA. Then the solutions are added to the respective resin portions and immediately treated with 142 µmol of DIPEA. The coupling reactions are continued for 60 minutes at room temperature and afterwards rinsed as follows:

2x 45 seconds with DMA
1x 5 minutes with acetic anhydride:pyridine:DMA = 1:1:8 (v:v:v)
5x 45 seconds with DMA.

Example 6.3: Chain lengthening of the tag via individual amino acids (alternative to Example 6.2)

First the trityl groups of the tag linked irreversibly to the resin are removed by cleavage with 5 % formic acid in dichloroethane, as described in Example 6.2.

For 5 portions of 4.3 µmol each of tag component

1:	Trt-G (I)	129 µmol
	Trt-G (II)	129 µmol
2:	Trt-G (I)	129 µmol
	Trt-N (II)	129 µmol
3:	Trt-A (I)	129 µmol
	Trt-A (II)	129 µmol
4:	Trt-N (I)	129 µmol
	Trt-A (II)	129 µmol
5:	Trt-A (I)	129 µmol
	Trt-N (II)	129 µmol

The Trt-amino acids listed above in (I) are preactivated for c. 40 minutes in 142 µmol of HOBT and 142 µmol of DICD solution in DMA. Then the solutions are added to the respective resin portions and immediately treated with 142 µmol of DIPEA. The coupling reactions are continued for 60 minutes at room temperature and afterwards rinsed as follows:

2x 45 seconds with DMA
1x 5 minutes with acetic anhydride:pyridine:DMA = 1:1:8 (v:v:v)
5x 45 seconds with DMA.

Afterwards the Trt groups are removed as shown above and each of the Trt-amino acids

listed above under (II) are coupled as indicated above.

Example 6.4: Synthesis of the 2nd variable position of the ligand

Position Y is synthesised in general accordance with the introduction of Z (with respect to the different codings) (q.v. reaction scheme 2). This is done by mixing all samples of Example 6.2 or 6.3 and dividing them into 4 portions of equal size (4 Y portions).

In these 4 separate portions, each of the monomers possible at this position is attached as described in Example 6.1

Pool	Monomer	Amount	Coding
1	Fmoc-L-Pro-OH	76 µmol	DA
2	Fmoc-D-Pro-OH	76 µmol	GA
3	Fmoc-β-Ala-OH	76 µmol	AA
4	Fmoc-L-Asp(OtBu)-OH	76 µmol	AN

As described in Example 6.2 or 6.3, the unit of the tag belonging to the monomer is then attached.

Example 6.5 Synthesis of the 3rd variable position of the ligand

Position X is synthesised in general accordance with the introduction of Y or Z (with respect to the different codings) (q.v. reaction scheme 2). This is done by mixing all samples of Example 6.4 and dividing them into 5 portions of equal size (5 X portions).

In these 5 separate portions, each of the monomers possible at this position is attached as described in Example 6.1

Pool	Monomer	Amount	Coding
1	2-cyanobenzenesulfonyl chloride	76 µmol	DG
2	Fmoc-D-Phe-OH	76 µmol	GD
3	Fmoc-N-benzylglycine-OH	76 µmol	GF
4	Fmoc-β-Ala-OH	76 µmol	AA
5	acetic acid anhydride	76 µmol	AG

The coupling with 2-cyanobenzenesulfonyl chloride is done via a reaction with a solution of the sulfonylchloride in pyridine/DMA at room temperature; the coupling of acetic acid anhydride is done as described in Example 5.

As described in Example 6.2 or 6.3, the unit of the tag belonging to the monomer is then attached.

Example 7: Deprotection of the ligand

An appropriate number of resin particles of the library obtained in protected form (but a multiple of the component number) is subjected to the deprotection reactions commonly employed in peptide chemistry to remove the Fmoc and tert-butyl protective groups. To remove the Fmoc protective groups, the resin of Example 6.5 is again treated repeatedly with 20 % Pip/DMA (q.v. Example 2) and then, to remove the t-butyl and Pmc protective groups, with 95 % trifluoroacetic acid (5 % water + 2% ethanedithiol) for 40 minutes at room temperature. If the linkage of the ligand to the resin is intact, the functional groups in the ligand and of the tag are liberated in their entirety. Rinsing is then carried out as follows:

4x 45 seconds with DCE
4x 45 seconds with isopropanol
4x 45 seconds with isopropanol:H_2O = 1:1 (v:v)
5x 45 seconds with H_2O.

Example 8: Marking of thrombin with fluorescein

isothiocyanate is added to a solution of human thrombin (0.53 mg in 100 µl of borate buffer pH 8) (10 µl of a solution of 2.9 mg in 290 µl of DMSO). The reaction is continued for 60 minutes at room temperature and the reaction mixture is worked up over a gel chromatography column (Sephadex®-G25, 15x0,5 cm) (elution with 1M NaCl).

20 µl of the fluorescein-thrombin fraction (c. 3 % of the fraction volume) are diluted to 500 µl and measured photometrically at 495 nm. This gives a fluorescein concentration of c. 2 µm. The fluorescein/thrombin ratio is thus about 1:1.

Example 9: Identification of thrombin-binding ligands in heterogeneous phase

About 500 beads of the resin of Example 7 are incubated in a 2.2 µm solution of

fluorescein-marked thrombin of Example 8 for c. 10 minutes at room temperature. After rinsing for 3x1 minute with a solution of:

68 mg of imidazole
876 mg of NaCl
147 mg of $CaCl_2x2H_2O$
3.35 g of PEG (3350)
ad. 100 ml of H_2O

the beads are examined visually in longwave light (blue light at c. 470 nm) for inherent fluorescence. Clearly fluorescing particles are sorted and rinsed

5x 3 minutes with formamide and
10x with H_2O

and subsequently dried.

Example 10: Assay for thrombin inhibition of the ligands of individual beads in liquid phase

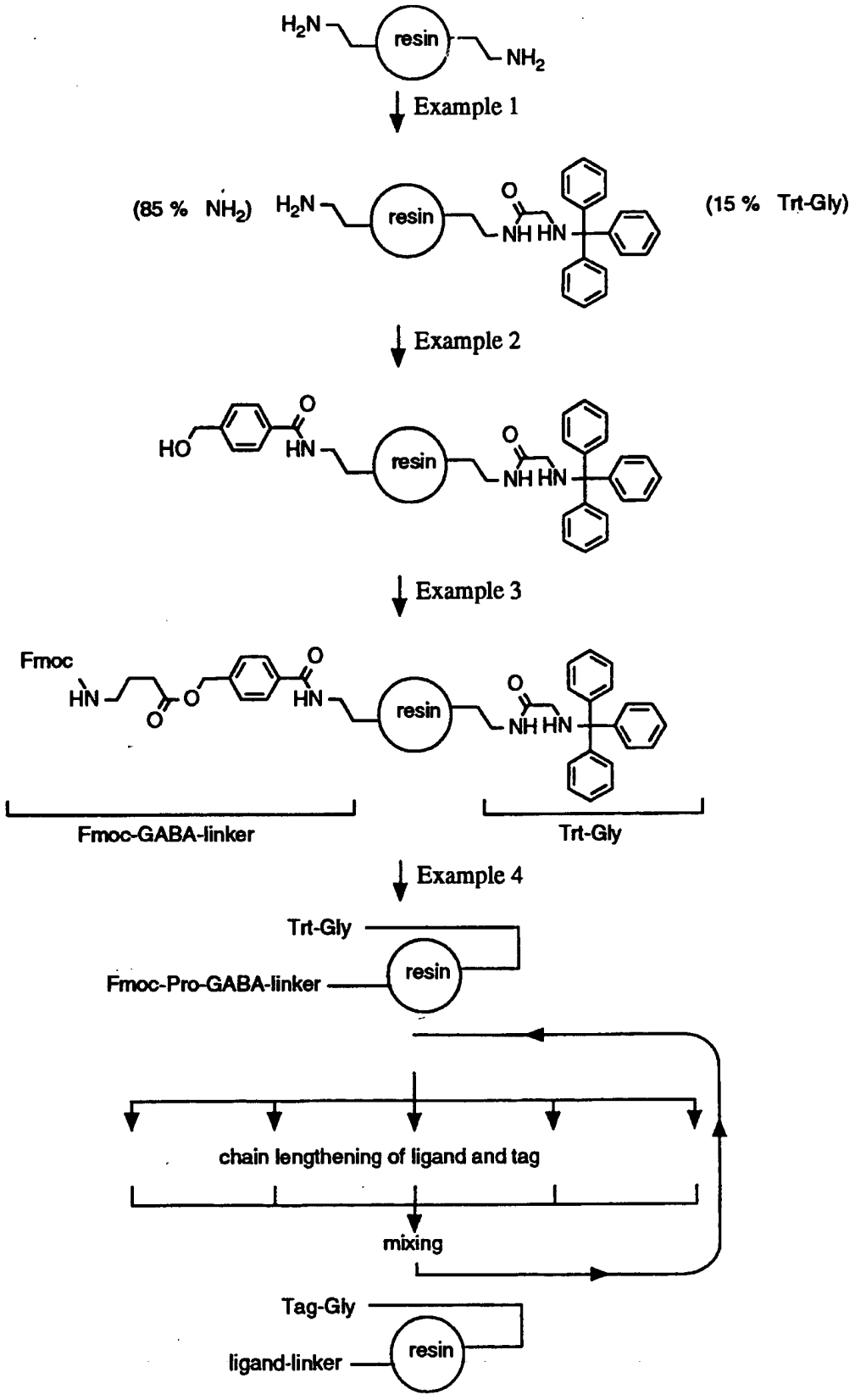
The beads sorted in Example 9 are kept in a saturated solution of ammonia in THF for 24 h at room temperature. Afterwards they are distributed individually into the numbered cavities of a microtitration plate with filter plates (e.g. a Millipore MultiScreen DV96®; pore size 0.65 µm). The ligands can be rinsed with a mixture of tetrahydrofuran/water into a second numbered microtitration plate, where they are assayed direct in a thrombin inhibition assay. Per cavity, $2.3x10^{-2}$ NIH units of thrombin and 9.3 µg of chromogenic substrate S-2302 (D-Pro-Phe-Arg-p-nitroanilide 2HCl; ex Chromogenix) are added in a final volume of 150 µl. The color development over a period of c. 75 minutes is measured with the aid of a Multiwell-Platereader at a wavelength of 405 nm, and the corresponding extinction values are plotted as a function of the time. Cross-comparisons of the gradients of diagrams of different inhibitors act as basis for identifying ligands having inhibitor activity.

The composition of two such identified thrombin-inhibitors is identified from the tag. This is done by screening each of the tags of the beads belonging to each ligand separated by solid-phase protein sequencing (Edman degradation) in an automated sequencing apparatus (ABI 477 available from Applied Biosystems), and identifying the composition of the two ligands from the code table as

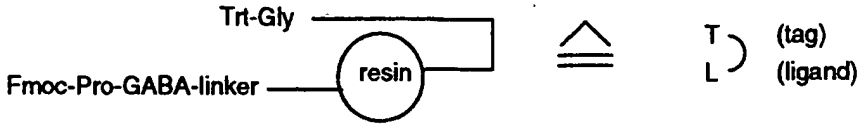
D-Phe-D-Pro-Arg-Pro-GABA
and
D-Phe-Pro-D-Arg-Pro-GABA

Molecular weight (MALDI-MS $M+H^+$) in each case 600.7

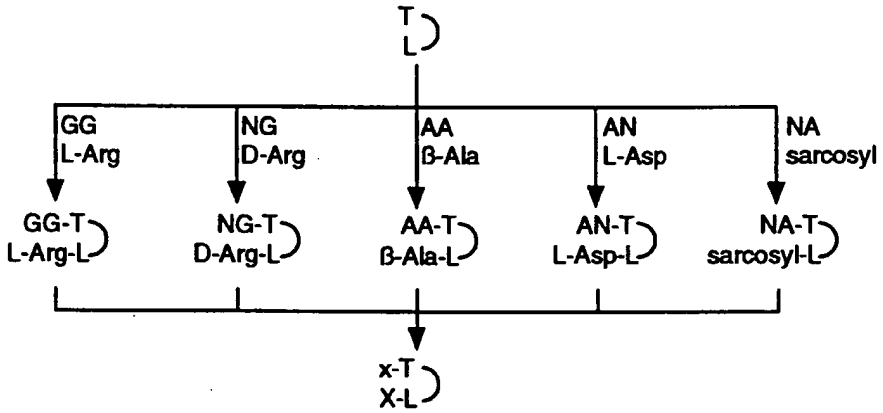
Reaction scheme 1:



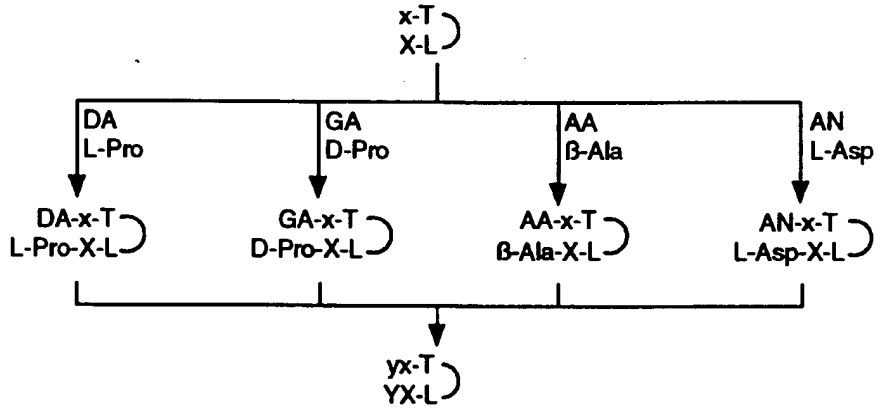
Reaction scheme 2:



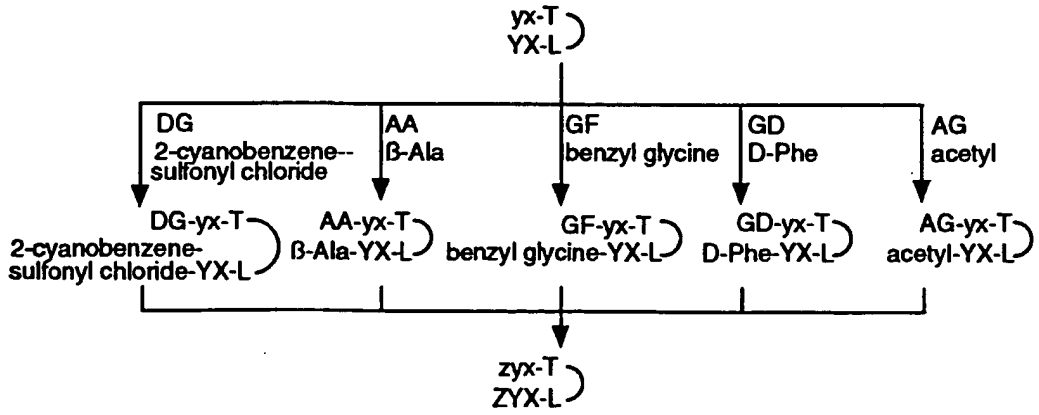
Introduction of the 1st variable monomer into the ligand and of the unit coding for it into the tag:



Introduction of the 2nd variable monomer and of the 2nd unit coding for it:



Introduction of the 3rd variable monomer and of the 3rd unit coding for it:



What is claimed is:

1. A library comprising a plurality of different units each consisting of a solid or semisolid carrier (beads), a synthetic oligomer (ligand) and an identification structure (tag) by means of which the monomers of the ligand can be indentified, wherein:

a) each carrier unit carries only one type of ligand,
b) ligand and tag are attached to the same carrier at different positions,
c) the ligand, irrespective of the tag and without altering its information content, can be separated from the carrier,
d) the tag is a sequenceable polypeptide, and
e) to synthesise the tag or ligand a protective group is used that can be removed under mild acid conditions.

2. A library according to claim 1, wherein the protective group which is removable under mild acidic conditions is at least 10x more labile than the dimethyldimethoxybenzyloxy-carbonyl protective group.

3. A library according to claim 1, wherein the protective group which is removable under mild acidic conditions is a group of the trityl type.

4. A library according to claim 1, wherein the ligands contain monomers which, in the amounts in which they are obtained on a carrier unit, cannot be clearly identified by known and/or automated procedures.

5. A library according to claim 1, wherein the ligand is attached to the carrier through a cleavable linker.

6. A library according to claim 1, wherein the ligand is attached to the carrier through a linker which is cleavable under basic, acidic, photolytic, oxidative or reductive conditions.

7. A library according to claim 1, wherein the ligand is attached to the carrier through a linker which is cleavable under basic conditions.

8. A library according to claim 1, wherein the ligand can be removed in several portions from the carrier.

9. A library according to claim 5, wherein the linker is selected from the group consisting of p-hydroxymethylbenzoic acid, 4-hydroxymethylphenylacetic acid, benzhydrylamino, allyl, hydroxycrotonylaminomethyl, 3-nitro-4-hydroxymethylbenzoic acid, p-nitrobenzhydrylamine, 4-[4,4'-bis(methylsulfinyl)-2-oxybenzhydrylamino]butyric acid and disulfide linkers.

10. A library according to claim 5, wherein the linker is 4-hydroxymethylbenzoic acid.

11. A library according to claim 1, wherein each monomer of the ligand is coded by more than one amino acid.

12. A library according to claim 1, wherein the tag is provided with a constant starting sequence.

13. A library according to claim 1, wherein the tag is provided with a constant end sequence.

14. A library according to claim 1, wherein the ligand is provided with a constant starting sequence.

15. A library according to claim 1, wherein the solid or semisolid carrier is a polymeric resin.

16. A library according to claim 1, wherein the polymeric resin contains free amino groups.

17. A library according to claim 1, wherein the ratio of ligand to tag on a carrier is greater than 1.

18. A library according to claim 1, wherein the ratio of ligand to tag on a carrier is from 2 to 100.

19. A library according to claim 1, wherein the ratio of ligand to tag on a carrier is from 4 to 10.

20. A process for the preparation of a library as claimed in claim 1, which comprises

synthesising ligands and tags by an orthogonal and alternating synthesis.

21. A process for the preparation of a library according to claim 20, which comprises the steps

a) attaching the first unit of the tag and the first monomer of the ligand or the building block thereof or a linker for each to the solid or semi-solid carrier;

b) optionally attaching further invariable monomers to the ligand or further non-variable coding units to the tag;

c) dividing the solid or semisolid carrier into portions for the variable monomers of the ligand;

d) carrying out in each portion separately, in codable sequence, further modifications at the ligand or attaching another of the variable monomers possible at this site of the ligand as well as the unit of the tag coding for this step;

e) mixing the portions;

f) repeating steps b) to e) until the variable part of the ligands is completely synthesised; and

g) optionally attaching one or more than one further non-variable monomer to the ligand or further non-coding units to the tag;

using for the synthesis of tag or ligand a protective group which can be removed under mild acidic conditions.

22. A process according to claim 20, wherein orthogonal protective groups are used for synthesising the ligand and the tag.

23. A process according to claim 20, wherein two groups of protective groups are used for synthesising the ligand and the tag, the first group being removable under mild acidic and the other group under basic conditions, by the action of light, or under oxidative or reductive conditions.

24. A process according to claim 20, wherein two groups of transient protective groups are used for synthesising the ligand and the tag, the first group being removable under mild acidic and the other group under basic conditions.

25. A process according to claim 21, wherein one of the protective groups is of the trityl type (Trt).

26. A process according to claim 21, wherein protective groups of the fluorenylmethoxy-carbonyl type (Fmoc) and of the trityl type (Trt) are used.

27. A process according to claim 19, wherein the synthesis of the ligand is carried out using Fmoc protective groups.

28. A process according to claim 19, wherein the synthesis of the tag is carried out using trityl protective groups.

29. A process according to claim 19, wherein the reactive side-chains of the monomers of the ligand and of the tag which shall not participate in the reactions are protected by a third group of protective groups.

30. A process according to claim 19, wherein the reactive side-chains of the monomers of the ligand or of the tag which shall not participate in the reactions are protected by pentamethylchromanesulfonyl (PMC), Mtr or protective groups of the tert-butyl type.

31. A process for detecting structures, which comprises treating a library as claimed in claim 1 with the acceptor to be investigated and isolating the components of the library that bind the acceptor.

32. A process according to claim 31, wherein the acceptor is a receptor, a transport protein, an antibody, an enzyme or a fragment thereof.

33. A process according to claim 31, wherein the acceptor carries an identifiable group.

34. A process according to claim 33, wherein the identifiable group is a fluorescing, chemoluminescing or radioactive group, avidine, biotine, a reporter enzyme or an immunologically detectable group.

35. A process according to claim 33, wherein the identifiable group is a fluorescing group.

36. A process according to claim 31, wherein the library as claimed in claim 1 is mixed with an acceptor that carries a fluorescing group, washed, and the components of the library that show fluorescence are isolated.

37. A process according to claim 31, wherein the ligands are separated wholly or partially from the isolated carriers and again subjected to an assay.

38. A process according to claim 37, wherein the further assay cannot be carried out with the ligands bound to the solid or semisolid carrier.

39. A process according to claim 37, wherein the further assay is a binding study, NMR or MS.

40. A process according to claim 37, wherein the ligands are separated from the isolated carriers by treatment with a readily volatile or gaseous agent.

41. A process according to claim 37, wherein the ligands are separated from the isolated carriers by treatment with an acid or an alkali.

42. A process according to claim 37, wherein the ligands are separated from the isolated carriers by treatment with an alkali.

43. A process according to claim 37, wherein the ligands are separated from the isolated carriers by treatment with gaseous ammonia, liquid ammonia or a solution of ammonia/THF.

44. A process according to claim 37, wherein a first portion of the ligands is separated from the isolated carriers by treatment with gaseous ammonia, and a second portion by treatment with a solution of ammonia/THF or liquid ammonia.

45. The compound of formula D-Phe-D-Pro-Arg-Pro-GABA or D-Phe-Pro-D-Arg-Pro-GABA, or a pharmaceutically acceptable salt thereof, for inhibiting thrombin.

46. A pharmaceutical composition comprising the compound of formula D-Phe-D-Pro-Arg-Pro-GABA, D-Phe-Pro-D-Arg-Pro-GABA, or a pharmaceutically acceptable salt thereof, and further optional excipients.

INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/EP 94/03936

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/68 C07K1/04 C07K17/00 G01N33/86 //G01N33/543, C12Q1/68,G01N33/566,G01N33/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PEPTIDE RESEARCH, vol.6, no.3, 1993, NATICK, MASS pages 161 - 170 NIKOLAIEV ET AL. 'Peptide-encoding for structure determination of nonsequenceable polymers within libraries synthesized and tested on solid-phase supports' see the whole document ---	1-46
A	EP,A,0 498 508 (VAN NISPEN) 12 August 1992 see examples --- -/--	45,46

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search	Date of mailing of the international search report
16 February 1995	27.04.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Ceder, O

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INTERNATIONAL SEARCH REPORT

Interna al Application No
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